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UTILISATION OF BEETROOT EXTRACT AS COLOURING AGENT FOR SHRIKHAND

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ABSTRACT

The colour is a very important physical characteristic of food products and has considerable impact on marketing of the products. Mostly, artificial i.e. synthetic colours are being used as colouring agents in several food products. Consumption of artificial colours reportedly is not advisable as they are purported to cause harmful effects when consumed in higher doses, whereas natural colors are not only safe for consumption but also provide health benefits like antioxidants to the consumer. In this project, beetroots (*Beta vulgaris*) which are rich in red pigment were extracted with water and the beetroot colour extract (BCE) was used as a coloring agent at 1, 2, and 3 % levels (on chakka basis) for shrikhand preparation. A 2% beetroot extract per 100 g on chakka basis gave pleasant pink colour to shrikhand. The stability of colour during storage of shrikhand was evaluated by sensory evaluation and also by measuring lightness, red, blue and green values by scanning method. The colour stability study indicated that the colour was stable during storage of shrikhand. The optimized level of beetroot extract had no significant impact on body and texture and flavour of shrikhand. Hence, the extract was recommended as colouring agent for shrikhand.

Keywords: Beetroot, Beetroot colour extract, shrikhand, storage, colouring agent

INTRODUCTION

Synthetic colours are widely used as colouring agents in foodstuffs, but colours derived from natural sources have their own consumer appeal. Several plant, animal and microbial materials act as sources of natural colours. Plant sources include leaves, fruits, roots, stem and vegetables. Beetroot (*Beta vulgaris*), an underground root with about 60 days of harvest time, is a popular vegetable throughout the world (but not a major vegetable). Its typical yield is 15 - 20 tons per acre of land. It is known for its dark red or purple colour or pigment popularly believed to enhance blood production when consumed. Several beetroot varieties exist, among those the most outstanding ones are

the red (beetroot, generally used for human feeding) and the white beet (sugar beet, used for animal feeding). Beetroot comes in different colour shades, however red one is the most popular. There are several varieties of beetroot viz. Pablo, Bonel, 'Nero, Favorit, Rubin' and 'Detroit [1]. Out of these, it was reported that the beetroot variety named as 'Pablo' variety produces a very intense pigment in the raw extracted beetroot juice [2]. There are three red coloured pigments in beetroot extract viz. betalamic acid, betaxanthin (yellow) and betacyanin (red). Their contents are 9.9 mg/100g, 16.3 mg/100g and 30.9 mg/100g beetroot pulp, respectively [3]. These three pigments are known as betalains and betanin accounts for 75-95% of betacyanin.

The beetroot has composition of: water 87.5%; fat 0.17%; protein 1.61%; carbohydrates 9.16%; and fibre 2.8% [4]. The nutritional value of beetroot juice is very high due to its high content of carbohydrate, folate, fiber, iron, nitrate, manganese, potassium, vitamin C and also free fat, low in calories, inexpensive and moreover beets are available throughout the year.

Consumption of beetroot is purported to have several health benefits, which are: improve oxygenation during athletic activities, tumour reduction, decrease the risk of obesity and overall mortality, diabetes, heart disease and promotes a healthy complexion and hair, increases energy, overall lowers weight and reduces chronic inflammation [6, 7]. Some research findings suggest that betanin is an effective cancer chemopreventive agent in mice when used at very low doses [8]. Betalains exhibit strong antioxidant activity [9]. However, the major disadvantage of beetroot colour is its low heat stability [5].

Shrikhand is a fermented, sweetened dairy product popular in western parts of India and is prepared from chakka. It originated in Maharashtra, and has a firm, smooth and spreadable consistency. Its flavour is because of diacetyl produced by lactic acid bacteria and many times combined with cardamom flavor and nuts. Mango pulp is also used as flavouring agent. Popular flavours used in shrikhand preparation are: cardamom, saffron, and almond, mango. Some manufacturers incorporate several other flavours like strawberry, chocolate etc. but they are popular only in localised places. Shrikhand has live lactic acid bacteria in the order of 10^4 - 10^6 per g . Amul dairy sells pasteurized shrikhand i.e. the product is subjected to heat treatment of at least $60 - 70^{\circ}$ C [10]. In this case, it is devoid of live lactic acid bacteria because they are killed by the heat treatment, but the benefit incurred is longer shelf life of shrikhand up to six months at refrigerated temperature. Shrikhand has composition of : moisture: 40%, sugar 42%, fat 5%, protein 6%, and lactose 1.6% [11].

Betalains have several applications in foods, such as gelatines, desserts, confectioneries, dry mixes, poultry, dairy (ice cream and yoghurt), and meat products; fortified milk product [12], cheese [13, 14], flavoured milk, yoghurt [15], yoghurt dip [16], biobeverage [17], strawberry flavoured ice cream [18], probiotic products [19, 20], flavoured milk and lassi [21,22]. In the present project, beetroot extract was used as colouring agent in shrikhand to get an attractive pink colour.

MATERIALS AND METHODS Materials

Fresh cow milk was procured from Cattle Yard of the Institute. Sugar and fresh beetroots were procured from the local market from a single source throughout the study period. The starter culture (mixture of Lactococcus sp) used for preparation of dahi required for chakka making was collected from the Dairy Chemistry and Dairy Bacteriology Section of the Institute. Polypropylene cups used for packaging shrikhand were purchased from the local market.

Experimental methods

Extraction of colour from beetroot

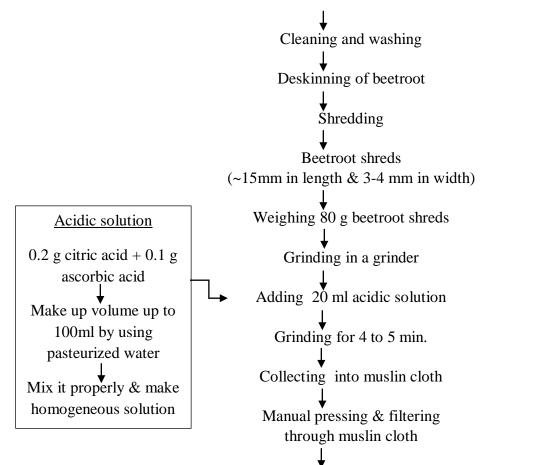
The beetroot extract was prepared by an optimized process and was termed as beetroot colour extract (BCE) (vide Flow chart). The BCE (Fig.1) was used as a colouring agent at different levels in shrikhand to get pink colour.

Preparation of shrikhand

Cow milk was standardized to 4 % fat and 8.5 % MSNF. It was heated to 90°C for 5 min and cooled to 40°C. Dahi culture was added @ 2%, mixed properly and incubated at 37°C for 24 h. The curd formed was collected in a muslin cloth and hung for 18 to 20 h in atmospheric condition to drain out the whey from curd and form a high acidic chakka (pH 3.7 to 3.8). Powdered sugar and BCE @ 50% and 1 to 3 % of chakka respectively on weight basis were added and mixed thoroughly in a planetary mixer (Lalith Industries, Bengaluru) into a homogeneous, smooth mass i.e. shrikhand. The shrikhand was packed in 100 ml

polystyrene cups and stored in refrigerated condition (5-7°C). The shrikhand was analysed for the following: Sensory evaluation parameters: colour and appearance, body and texture, flavour and overall acceptance on a 9point Hedonic scale; Colour parameters: Red, Green, Blue and Lightness values by Scanner – Adobe Photoshop method; Chemical parameter: pH.

Medium size of beetroot



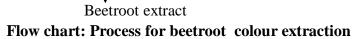






Fig.1 : Beetroot colour extract (BCE)

Stability of beetroot extract in shrikhand during storage

Shrikhand samples added with beetroot colour extract (BCE) and packed in polystyrene cups were stored at 5-7°C and monitored at frequent intervals to study stability of colour intensity and shelf life. The color stability was checked by scanning of product and measuring the colour parameters at frequent intervals until the products spoiled. The products were also sensorily evaluated for various sensory attributes.

Analytical methods

Colour measurement

Colour of the samples was measured by Scanner-Adobe Photoshop (SAP) method [23]. All the samples were tempered to about 30°C before measurement. With the help of a spatula, shrikhand was scooped into petri plate and spread uniformly by evening the surface. Any air gaps were collapsed by spatula. The petri dish containing the sample was placed on the bed of the scanner and was covered by scanner top. Scanning was performed and the image was saved as JPEG file. Scanning of the sample was performed using scanner [Hewlett-Packard Scan jet 5370c (hp)] under the following scanner parameters: resolution: 75 dpi, sharpness: medium; image quality: medium; background of the sample: white; thickness of sample: 1 cm. Colour analysis software namely Adobe

Photoshop Version CS3 running under the Microsoft Windows XP environment (Computer: Dell, Core-i3, 4GB RAM) was used to extract and analyse colour information from the scanned image. The scanned image in JPEG format was opened in Adobe Photoshop and the following parameters were measured in RGB mode: Lightness (L), red (R), green (G) and blue (B).

Sensory evaluation

Shrikhand is normally consumed at refrigerated temperature, hence the shrikhand samples were evaluated for sensory acceptance at refrigerated temperatures. The main objective of the sensory evaluation was to find out whether added beetroot colour extract (BCE) imparted its any flavour to the product and whether its addition has any adverse or favourable influence on other sensory qualities of products.

Shrikhand samples were served in polystyrene cups in which they were packaged earlier. The sensory evaluation sessions were conducted inside laboratory by interactions among the judges. The panel of judges consisted of semitrained faculty members as well as postgraduates who showed keen interest and aptitude towards sensory evaluation. The judges were asked to evaluate colour and appearance, body and texture. flavor and overall acceptability of the samples on a 9-pont Hedonic scale (9 - like extremely, 1 - dislike

extremely) [24]. Plain water was provided to rinse the mouth in between the samples. Sensory evaluation sessions were organized around 12 noon or 3-5 PM every time.

pH of shrikhand

pH of shrikhand was determined by dipping the electrode of pH meter (Eutech Instruments, Bengaluru) directly into the product taken in an appropriate container at ~27°C.

Microbial analysis of beetroot extract added shrikhand

The prepared beetroot extract added shrikhand was analysed for total plate count and yeast & mould count during storage following the methods mentioned for milk in BIS [25].

Statistical

All experiments were conducted in one way randomized block design. The sensory

evaluation data and colour parameters' data were subjected to one way ANOVA using SPSS software version 16.0. The significance of treatments' effect was determined by Tukey test.

RESULTS AND DISCUSSION

Effect of BCE level on the quality of shrikhand

Chakka was prepared as previously described and added with beetroot colour extract (BCE) @ 1, 2, 3 and 4ml per 100 g during the stage of mixing of sugar. The BCE produced a pleasant pink colour in shrikhand (Fig.2). The product which was not coloured (plain shrikhand i.e. control sample) scored colour and appearance (CA) score of 7.51 whereas that added with 1 ml BCE scored 7.68 which was not statistically significant (P>0.05) (Table-1). However, with increasing BCE



Fig.2: Plain shrikhand (on left) BCE added shrikhand (on right)

ml BCE per 100 g chakka	СА	BT	Flavor	OA
Control (0)	7.51 ^a ±0.39	7.65 ^a ±0.	7.60 ^a ±0.43	7.60 ^a ±0.36
1	7.68 ^{ab} ±0.53	$7.61^{a}\pm0.48$	$7.62^{a}\pm0.39$	7.70 ^b ±0.37
2	$7.84^{b}\pm0.49$	$7.67^{a}\pm0.49$	7.73 ^a ±0.39	7.88 ^c ±0.39
3	$7.62^{ab} \pm 0.64$	$7.66^{a} \pm 0.51$	$7.7^{a}\pm0.48$	7.72 ^b ±0.49
F - value	2.561	0.110	0.767	2.699

Table-1: Effect of addition of BCE as colouring agent on the sensory scores of shrikhand

Note: CA-Colour and appearance, BT-Body and texture, OA-Overall acceptability BCE - beetroot colour extract

addition, the CA score increased. The CA score significantly increased to 7.84 on addition of 2 ml BCE while use of 3% did not further enhance the CA score. The body and texture (BT) scores showed that there was no adverse impact on BT of shrikhand. The BT scores were 7.65, 7.61, 7.67 and 7.66 for the product added with 0, 1, 2and 3 ml BCE (Table-1). These values were not statistically significant. Shrikhand being a viscoelastic solid, addition of liquid extract even up to 3 ml did not much affect its BT quality. Similarly, flavor quality was not affected as indicated by the flavor scores 7.60,7.62, 7.73 and 7.70 respectively for 0,1, 2 and 3 ml BCE containing shrikhand samples, which were not significantly different from each other (P>0.05). However, overall quality was significantly enhanced to 7.88 by addition of 2 ml BCE to shrikhand whereas the control sample was awarded a score of 7.60. In fact, use of 1 ml

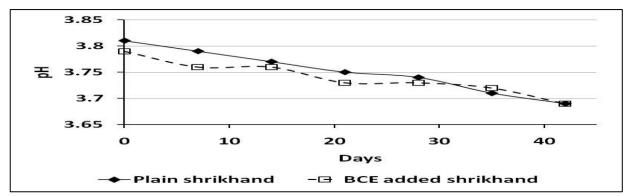
extract itself significantly enhanced the overall acceptance as it imparted pleasant pink colour to the product.

Stability of colour of BCE in shrikhand during storage at 5°C

The beetroot colour stability was studied during the storage of shrikhand. BCE was added to chakka during the preparation of shrikhand which was then packed in polystyrene cups and stored at 5°C. The colour parameters were measured during storage and tabulated in Table-2. It may be seen from the table that the initial RGB and L values of shrikhand without adding BCE were 253.11, 209.43, 192.67 and 223.86 and these changed to 254.94, 210.23, 200.74 and 222.86, respectively at the end of 0. 9, 18, 27, 36 and 45 days of storage. But these changes during storage were found to be statistically not significant (P>0.05). The shrikhand carried a

Days	Luminosity	Red	Green	Blue
0	223.86 ^a ±7.5	253.11ª±7.4	209.43 ^a ±12.2	192.67ª±7.5
9	219.91 ^a ±4.0	248.51 ^a ±3.4	210.70 ^a ±5.3	193.37 ^a ±9.2
18	$224.13^{a} \pm 10.8$	252.67 ^a ±10.1	214.76 ^a ±7.0	193.69 ^a ±9.5
27	$216.87^{a} \pm 11.7$	246.49 ^a ±12.9	207.64 ^a ±7.5	196.31 ^a ±10.3
36	222.34 ^a ±9.1	253.13 ^a ±9.4	207.62 ^a ±9.1	197.41 ^a ±6.4
45	222.86 ^a ±5.4	254.94 ^a ±8.9	210.23 ^a ±10.1	200.74 ^a ±6.2
F - value	0.320	0.376	0.268	0.409

Table-2: Effect of storage on colour parameters of BCE added shrikhand at 5°C



^{*} Measured under RGBL mode on a scale of 0-255

Fig. 3: Effect of storage on pH of BCE added shrikhand at 5°C

(Shrikhand added with 2ml BCE per 100 g chakka)

pleasant pink colour throughout the storage period. The stability of colour was aided by low pH which was maintained throughout the storage as indicated in Fig.3. The pH of shrikhand added with BCE was 3.69 - 3.79whereas that for control sample was 3.69-3.81.

Effect of storage on sensory score of BCE added shrikhand at 5°C

The BCE added shrikhnad was packaged in polystyrene cups and stored at 5°C. In shrikhand, the beetroot extract produced a pink colour which was liked by the judges. This colour was

Days	CA	BT	Flavour	OA
0	$8.25^{a}\pm0.29$	$7.88^{a}\pm0.31$	8.00 ^c ±0.11	8.17 ^c ±0.24
9	$8.25^{a}\pm0.29$	7.88 ^a ±0.31	8.00°±0.11	8.17°±0.24
18	$8.25^{a}\pm0.29$	7.88 ^a ±0.31	8.00 ^c ±0.11	8.17°±0.24
27	$8.25^{a}\pm0.29$	7.88 ^a ±0.31	8.00°±0.11	8.17°±0.24
36	8.25 ^a ±0.29	$7.88^{a}\pm0.31$	$7.36^{b}\pm0.20$	7.27 ^b ±0.15
45	8.25 ^a ±0.29	$7.88^{a} \pm 0.31$	$5.19^{a}\pm0.89$	5.14 ^a ±0.67
F - value	0.000	0.000	128.12	187.20

Table-3: Effect of storage on sensory score of BCE added shrikhand at 5°C

Note: All scores on 9 – point Hedonic scale; CA-Colour and appearance, BT–Body and texture, OA- Overall acceptability; BCE - beetroot colour extract

stable throughout the storage as indicated by same CA scores (8.25) (Table-3). As reported earlier, beetroot colour is more stable at low pH levels. Since shrikhand has a low pH, this might have aided in stability of the beetroot colour. The beetroot colour showed no influence on body and texture of shrikhand as the BT score was 7.88 throughout the storage. However, the same was not the case with flavor. The flavor score initially was 8.00 which remained the same up to 27 days indicating that the flavor was maintained well, but thereafter there was slight significant decrease (7.36) attributable to development of acidity. After 45 days, the flavor score reduced to 5.16 as a result of sourness of the product. Thus, it was concluded that shrikhand added with beetroot extract remained well up to 36 days at 5°C and thereafter it developed sourness leading to unacceptable scores. There was no statistically significant change in CA and BT scores, but significant change in flavor and OA scores. Prajapati *et al.* [26] reported a shelf life of 45 days at 10°C for shrikhand.

Microbial analysis of BCE added dairy products

Shrikhand added with beetroot extract was stored at 5°C and evaluated for microbiological quality. The purpose of this study was to observe any impact of beetroot extract on the microbial counts of shrikhand under study during storage. The initial total plate count (TPC) count was 9.63 log count per g (Table-4). This increased to 9.67 log counts per g at the end of 42 days of storage. The corresponding values for BCE containing product were 9.40 and 9.87 log cfu per g. It may

Dava	Total plate count, log cfu/g		Yeast and mold count, log cfu / g		
Days	Control	BCE added sample	Control	BCE added sample	
0	9.63	9.40	<10	<10	
42	9.67	9.87	<10	<10	

Table-4. Effect of storage on microbial counts of DCE added products	Table-4: Effect of storage of	on microbial counts	of BCE	added products
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Note: BCE - beetroot colour extract

be observed from these values that there was not much difference in the TPC counts of control sample and beetroot extract containing samples indicating that BCE had no influence on the LAB growth during storage. However, there are reports that extracts from beetroot pomace exhibited antibacterial activity against Salmonella typhimurium, Citrobacter freundii, Staphylococcus aureus, Staphylococcus sciuri and Bacillus cereus organisms [27]. Recent studies have shown that betanines have antioxidant, antimicrobial and antiviral activity [28]. In the present study, no such effect was observed. This further means that beetroot extract can safely be added to milk before fermentation itself so that the same colour level and pH is expressed in the final product also. With regard to yeast and mold count, the count remained below 10 per g throughout the storage period both in control as well as BCE containing products.

CONCLUSION

Beetroot extract can be used to colour shrikhand samples to get an attractive reddish pink shade.

This will enhance the consumer acceptance as well as nutritional appeal. The color is stable during the storage period of 45 days. This colour will go well with strawberry flavoured shrikhand.

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